

## PRODUCTION IN BACTERIA AND YEAST OF HEMOGLOBIN AND ANALOGUES THEREOF

### ABSTRACT

The alpha subunits of hemoglobin, which in nature are formed as separate polypeptide chains which bind noncovalently to the beta subunits, are here provided in the form of the novel molecule di-alpha globin, a single polypeptide chain defined by connecting the two alpha subunits either directly via peptide bond or indirectly by a flexible amino acid or peptide linker. Di-alpha globin may be combined in vivo or in vitro with beta globin and heme to form hemoglobin. Di-alpha globin is expressed by recombinant DNA techniques. Di-beta globin may be similarly obtained.

We further describe the production of tetrameric human hemoglobin and di-alpha/beta<sub>2</sub> hemoglobin in the yeast *Saccharomyces cerevisiae*. The synthesis of the protein is directed by a synthetic promotor consisting of two functional parts, an upstream activator sequence (UAS) that confers inducible transcription by galactose from a consensus yeast transcriptional initiation site. The expression construct is designed such that translation is expected to initiate at the same position as the human wild-type genes for  $\alpha$ - and  $\beta$ -globin. Three different types of expression vectors have been used: (1)  $\alpha$ -globin and  $\beta$ -globin contained on two separate plasmids (pGS4688 and pGS4988) in a diploid yeast strain; (2)  $\alpha$ -globin and  $\beta$ -globin each contained on a single plasmid (pGS289 and pGS389) and expressed in either haploid or diploid strains; and (3) di-alpha-globin and beta globin contained on a single plasmid (pGS 3089) and expressed in haploid strains.

Finally, we describe the co-expression of alpha and beta globin chains are co-expressed from a constructed polycistronic operon, and are folded together and combined intracellularly with heme to form active tetrameric hemoglobin. The hemoglobin may be recovered from the cells' soluble fraction. The invention thus obviates the need to express alpha and beta globin separately, solubilize, renature and

ABSTRACT - CONTINUED

purify them, and combine them in vitro with heme to obtain an artificial hemoglobin. By way of comparison, the separately expressed beta globin known in the art is deposited in inclusion bodies.